DP Barcode: 380085 MRID No.: 48120501

DATA EVALUATION RECORD
HONEY BEE – FIELD TEST WITH COTTON

1. **CHEMICAL:** Dinotefuran PC Code No.: 044312

2. **TEST MATERIAL:** Dinotefuran 70 SG Purity: 70% a.i. (trial 1); 68.7% (trials 2 &3)

3. **CITATION**

Authors:	Hummel, R., PhD. (Landis International, Inc.)
Title:	Field evaluation of foraging honey bees (*Apis mellifera*) and hives after exposure to dinotefuran applied to cotton
Study Completion Date:	June 9, 2010
Laboratory (field study):	Landis International, Inc. P.O. Box 5126, 3185 Madison Highway, Valdosta, GA 31603-5126
Laboratory (residue studies):	Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD 21601
Sponsor:	Mitsui Chemicals Agro, Inc., Agrochemicals Division, 1-5-2 Higashi-Shimbashi, Minato-ku, Tokyo 105-7117, Japan
Sponsor Study ID:	43421F008
MRID No.:	48120501
DP Barcode:	380085

4. **APPROVED BY:**

Primary Reviewer: Tanja Crk Tanja Crk **Date:** 2.25.2011
Biologist, OPP/EFED/ERB3

Secondary Reviewer: Allen Vaughan Allen W. Vaughan **Date:** 3.2.2011
Entomologist, OPP/EFED/ERB5

Tertiary Reviewer: Thomas Steeger Thomas Steeger **Date:** 4.25.2011
Senior Advisor, OPP/EFED/ERB4

5. **GUIDANCE:**
OPPTS 850.3040 Field Testing for Pollinators

6. **STUDY PARAMETERS:**

Age of Test Organism at Test Initiation:	Full bee colonies (range of broods of all ages to adults)
Type of Concentrations:	Nominal (contact foraging)

Definitive Test Duration: *Ca.* 27 days (4 week exposure period); exposure period followed by non-treatment incubation area for overwintering – 3 trials each of varying duration (189, 216, and 182 days post-initial hive exposure to test area)

7. CONCLUSIONS:

Three separate trials were performed (over two years: July-Aug. 2008; July-Sept. 2009; August 2009). For each trial, twelve honey bee hives were prepared and maintained on new hive components. Two treated plots were approximately five acres each; one plot received two foliar applications of 40.5 g a.i./A, and the other plot received two foliar applications of 60.75 g a.i./A. A third plot in the same field was maintained as the non-treated control area; the amount of space between treatment and non-treatment units was not specified. Four hives were randomly assigned to each of three treatments, and placed into the center of the appropriate plot seven days following the final foliar application (non-treated hives were placed at the far edge of the non-treated cotton area).

Dinotefuran (initial application) was applied to cotton plants approximately two weeks prior to peak flowering period. The second application was applied one week prior to peak flowering. At the initiation of approximate peak flowering, *i.e.,* 1 week after the second treatment, four test hives were moved to the approximate center of each treated plot, where they were positioned in an area of approximately 9 by 9 meters that was cleared by mowing. The clearing was made accessible by mowing a 3-meter wide path from the edge of the field. A shade structure was erected in each treated plot to provide hives relief from direct sunlight.

All hives within a trial location were positioned in the assigned field one week after the second application of dinotefuran for a minimum of 27 days (*i.e.*, 4-week exposure period). After removal from the assigned field, all hives were maintained at a common location for the remainder of the study. A final evaluation of all hives was performed in February following initial exposure of the hives to the test area.

No statistically significant effects ($p>0.05$) were reported for any test parameter (*i.e.*, honey yield, adult population estimate, brood production, adult bee mortality, bee foraging vigor, Spring colony assessment) that could reliably be attributed to treatment. Apart from estimated number of capped brood effects in trial 1, 42 days post exposure (at control, 200g a.i./A, and 300g a.i./A of 2893, 3971, and 1252, respectively)[1], observed differences of treatment groups to controls appear to be due to variability and/or are not treatment related. In trial 3, for example, it appears that treatment groups indicated an increase in frame mass[2] by 78 days post exposure that was

[1] Compare to pre-treatment estimates of 5709, 8006, and 6779, respectively.

[2] Pre-treatment mass of frames (in g) for control, 200g a.i./A, and 300 g a.i./A groups was 14,738, 14, 713, and 14,763 g; but by 78 days after exposure the masses were 11, 275, 14,138, and 16,625g, respectively.

statistically different from the control. Statistical analysis was not performed by the primary reviewer because raw data were not submitted for endpoints of concern. Therefore, the results reported in this evaluation are based on the study author's calculations. In addition, statistical verification of the adult bee mortality was not possible as the raw data were not provided. Therefore, the statistical significance of what appears to be a dose-response effect on bee mortality in Trial 1 (p. 24 of study) could not be calculated.

Concentrations of dinotefuran residues appear to be higher in flower nectar compared to flower pollen. Residues were detected in nectar samples from treated flowers up to 27 days after application in trial 1 and up to 26 days after application in trials 2 and 3. These time points represent the latest time points that residues were collected from flowers in treated plots. The fact that residues were still present in these flower nectar samples, including one sample that had quantifiable residues of 0.013 ppm (LOQ = 0.004 ppm) after 26 days (trial 3), indicates that residues could potentially be found in nectar from extra floral nectaries in cotton plants as well as the flowers. These nectaries, which are not part of the blossom, may continue to produce nectar even after bloom that bees will use as a sugar source. Therefore, continual chronic exposure is possible. Furthermore, trial 2 shows that dinotefuran residues can be detected in stored honey inside hives at least 85 days after application, which also suggests potential long-term exposure inside the hive, which may have implications for overwintering success as well.

Although bees were exposed to dinotefuran, the dinotefuran residue levels detected in treatment plot samples of nectar, honey, beeswax, and pollen, did not frequently differ greatly from those detected in the control samples, which is indicative of cross contamination in the control plot blossoms and hives (of all three trials in pollen and/or nectar samples) and likely foraging of treatment groups on control plots and of control groups on treated plots. For example, only trial 1 samples of nectar in flowers for days 7 and 17 indicated a clear distinction between no residue detected in controls and detected levels (0.012-0.021 ppm) in both treatment plots; and, in trial 3 day 26 flower nectar samples where no residues were detected in the control, but were detected in the two treatments as 0.013 ppm and <LOQ (0.004 ppm). Since residues were detected in controls at quantities frequently similar to those of treated groups, the ability of the study to differentiate treatment effects is uncertain. In addition, the lack of quantitative data rather than simply presence/absence on overwintering parameters such as egg abundance, larval abundance, pupal abundance renders the study inconclusive in terms of assessing overwintering success. The data suggest that all colonies (control and treatment) for all three trials were healthy (w/ >4 frames of adults) by approximately 200 days after exposure (*i.e.*, Spring colony assessment performed in February for all three trials), with presence of queen, larvae, and eggs. However, these hive attributes were not quantified. Instead, the quantitative data for this time step indicates that all colonies had adults present in all 10 frames of each colony; this was the case for all three trials. Also, an estimate of the number of capped brood was only provided for trial 1, but the difference between control and treatment groups did not appear to be significant (based on study author calculations using Duncan's NMRT with α set to 0.05). In addition, presence of queens may not be an indicator of overwintering success as new queens were added to hives when queen

loss occurred during the study. While re-queening hives is a common management practice, it is not particularly desirable for field studies as it prevents a test from quantifying the colony's ability to supersede naturally.

The effects data from this study cannot be used for effects assessment purposes on account of the study design where treated and control bees were not sufficiently segregated to prevent dinotefuran residues from moving into control plots. Similarly, residue values below the LOQ (*i.e.*, a <LOQ value that was calculated by integration for samples where residue values were <0.010mg/kg) cannot be used quantitatively in the risk assessment; therefore, the residue analysis is of limited value other than characterizing qualitatively the presence/absence of dinotefuran residues over time. In addition, there are two major metabolites, MNG and DN, for which no residue data are provided in this study. The presence/absence data for residues, particularly in nectar, are important, however, because they suggest that residues could potentially be present in extra floral nectaries once flowers have been shed, and dinotefuran (or, total) residues will be present in stored food inside hives for long periods of time (*i.e.*, at least 85 days after application). Therefore, honeybee adults and brood will likely be exposed to low levels of dinotefuran for relatively long periods of time, but the toxicity of these levels of residues remains an uncertainty.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental, (qualitative use only)

B. Rationale: While no statistically significant ($p>0.05$) effects were observed by the study author that could reliably be attributed to treatment, the study author did not submit raw data and the statistics could not be substantiated. Furthermore, the study design does not allow for statistical comparisons. In each of the three trials, large fields were divided into 3 plots which were used as treatment units. Only one plot (ranging in size from 4.7 – 8 acres) per treatment level within one field (ranging in size from 18-30 acres) per year precludes any meaningful statistical comparison within and across trials. The study can be considered pseudo-replicated on account of four hives present in each plot. However, comparison of plots within a trial is not possible on account of having no true replicates for a given plot within a given trial, which implies that this is an un-replicated longitudinal study. Furthermore, each year represents a unique trial given different environmental conditions and field/hive management regimes. Therefore, comparison across trials is also not possible.

The data show that dinotefuran residues were sometimes detected in nectar and pollen from control plots (*e.g.* 0.011 ppm in flower nectar at 15 days post-application in trial 3 and detectable in flower pollen at 27 days post-application in trial 1). These detections suggest that the plots were so close in each field trial that the control plots may have been

exposed to drift from the spray applications in treated plots. Alternatively, it may be possible that control bees foraging on treated plots transferred pollen to control plots and/or hives. Control hive samples of pollen in every trial (detected at every time point) and nectar in every trial were contaminated with dinotefuran, even in the absence of detectable residues in the sampled flowers from control plots. Given detections of dinotefuran in hive nectar (plot 1) at day 34, may suggest that nectar was collected by bees from either available flowers or extrafloral nectaries (which are present, but may or may not be active, continuously – even when flowers fall off). Though dinotefuran detections were not made on the extrafloral nectaries of cotton plants, the presence of dinotefuran in flower nectar (e.g., up to day 27 post-application for plot 1 samples) lends strong support for dinotefuran concentrations in sugar supplied to extrafloral nectaries from the common phloem source for these nectar-producing parts and dinotefuran's systemic properties, which would have implications for long-term exposure post-bloom.

Many of the results for parts of the plants and hive that were sampled were below the level of quantitation; therefore, although detected the magnitude of the residues is not quantifiable. In addition, a non-guideline freezer storage stability study (MRID 48120502) did not provide details on the freezer storage conditions (temperature, etc.) and was subsequently classified as 'unacceptable, but upgradable.' This study would validate whether freezing the samples in -20°C (as reported in this field study MRID 48120501) had an effect on the stability of the samples. Nonetheless, these residue results suggest that the plots were too close together and allowed control bees to forage in treated plots and vice versa. Given the cross-contamination, the ability of the study to discriminate treatment effects is uncertain.

Other causes of concern in the study design are listed below:

- The study authors only state the limit of quantitation (LOQ), but they do not state the limit of detection. Furthermore, this particular study employs LC/MS/MS with a limit of quantitation at *0.01 ppm*, which is not sensitive enough to quantitatively assess exposure through contaminated food sources. Given the toxicity of dinotefuran on an acute oral basis (48-hour LD_{50} = 0.0076 µg a.i./bee, MRID 45639727), concentrations comparable (*i.e.*, *0.06 ppm*, where 0.0076 µg a.i./bee * [1 bee/0.128g] = 0.06 µg a.i./g of bee) to the LOQ (0.01 ppm) may be toxicologically relevant.
- Bees may have used alternative forage sites since cotton is not a good pollen (protein) source, though it is a very good nectar (carbohydrate) source. In addition, all hives within a trial location were positioned in the assigned field for a minimum of 27 days (*i.e.*, 4-week exposure period). It is possible that bloom was in successive decline over the exposure period such that suitable forage for the bees was also reduced.
- Colonies are potentially fairly young as new foundation and new boxes were used.

It appears that frame foundations were constructed close to study initiation in the Spring, so it is unclear how long the bees had to equilibrate (the study authors indicate that hives were provided with new foundation stock several weeks before test initiation). Typically, 6-8 weeks are required for package bees to equilibrate in their colonies to lay down successive brood; otherwise, the bees may exert more energy building up the colony rather than laying down stores for the test duration.

- The colonies were treated for disease/pests; however, these treatments apparently were on an as needed basis and were not uniform across study colonies.
- The treatment units were treated with a variety of compounds including aldicarb (Temik), which is highly toxic to honeybees; however, residue analyses beyond those for dinotefuran were not provided. A full spectrum residue analysis (on pollen and nectar) would have been preferred to account for additional contaminants and metabolites/degradates of dinotefuran.
- Study authors intentionally destroyed queen cells to prevent swarming. Typically, additional frames are added to allow colonies to expand and thus prevent swarming. The presence of queen cells may be an indicator of colony (queen) performance as bees will not typically attempt to supersede the queen if she is functioning normally and conditions are not crowded in the colony. The fact that several hives swarmed suggests that the colonies were not sufficiently monitored and accommodated.
- New queens were added to hives when queen loss occurred during the study. While re-queening hives is a common management practice, it is not particularly desirable for field studies as it prevents a test from quantifying the colony's ability to supersede naturally.
- Reliance on a flat sheet of fabric (5 x 6 ft) placed on the ground in front of the hive to monitor adult bee mortality may result in counts that are negatively biased as dead bees may be removed by scavengers. Dead zone dead bee traps (boxes fitted with screens) would have been a better method to employ since they prevent bees and scavengers from removing dead bees from these observation areas.
- Removing all of the bees from the hive periodically through the study (*i.e.*, right after placement to and before removal from the test site, at 42-50 days, and 65-78 days) by shaking the frames into a large funnel to get an estimate of adult bee mass is likely a major stress factor.
- Trial 1 may have been compromised by the 7.6 inches of rain which fell during an August tropical storm event. Since the compound is systemic, presumably a significant portion of the amount applied roughly one month earlier had been taken up into the plant; however, bees would not have foraged on the treated plants for about two days.
- Pretreatment analysis of hive vigor for trial 2 indicated that there was a difference in the estimated number of capped brood between the control hives and the 300 g ai/ha treatment group. This pretreatment difference may affect any future comparisons hive vigor through the study period.

- The management practices in the "incubation period" were not described. This period immediately followed the exposure period after which the hives were evaluated for overwintering success. These management practices should be characterized to evaluate the environmental conditions to which the hives were exposed.

C. Repairability: Not Upgradable

9. SUBMISSION PURPOSE: This study was submitted to determine the potential effects of dinotefuran formulation 70 SG to honey bee colonies (*Apis mellifera* L.) in cotton fields in Georgia, USA. Potential effects on bee brood development were a primary focus.

10. MATERIALS AND METHODS:

A. Test Organisms

Parameters	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera* L.
Age at beginning of test:	Varied; entire colonies tested

Parameters	Reported Information
Colony composition	• *ca.* 18,984 to 25,434 bees per colony (estimated mean population) at the first assessment day (*i.e.*, pre-treatment hive vigor) • hives constructed of new materials in the Spring before study initiation (to prevent pesticide carry-over from previous years) • each test hive was headed by naturally mated queens of the same lineage and same approximate age • to prevent swarming, new queen cells were destroyed when found during the study • hives were selected from a successfully overwintered group • described as appearing healthy at initiation of each trial (3 trials total) • hive diseases and parasites were monitored and managed according to local recommendations
Supplier:	Wilbanks Apiaries (Statesboro, GA)
All bees from the same source?	Yes, same apiary. Hives were supplied and managed by Wilbanks Apiaries (Statesboro, GA)

Bee hive placement throughout the study:

Prior to transport to the test fields, test colonies were equalized by swapping frames, where necessary, to ensure that all hives within a trial location start with equivalent amounts of food stores, brood, and adults.

Three separate trials were performed (over two years: July-Aug. 2008; July-Sept. 2009; August 2009). For each trial, twelve honey bee hives were prepared and maintained on new hive components. Two treated plots were approximately five acres each; one plot received two foliar applications of 40.5 g a.i./A, and the other plot received two foliar applications of 60.75 g a.i./A. A third plot in the same field was maintained as the non-treated control area. Four hives were

randomly assigned to each of three treatments, and placed into the center of the appropriate plot seven days following the final foliar application (non-treated hives were placed at the far edge of the non-treated cotton area).

Dinotefuran (initial application) was applied to cotton plants approximately two weeks prior to peak flowering period. The second application was applied one week prior to peak flowering. At the initiation of approximate peak flowering, *i.e.,* 1 week after the second treatment, four test hives were moved to the approximate center of each treated plot. An area of approximately 9 by 9 meters was cleared by mowing. The clearing was made accessible by mowing a 3-meter wide path from the edge of the field. A shade structure was erected in each treated plot to provide hives relief from direct sunlight.

All hives within a trial location were positioned in the assigned field for a minimum of 27 days (*i.e.*, 4-week exposure period). After removal from the assigned field, all hives were maintained at a common location for the remainder of the study. A final evaluation of all hives was performed in February following initial exposure of the hives to the test area.

B. Test System

Parameters	Reported Information			
Test system	• Four beehives were placed in the middle of the respective treatment plots; non-treated hives were placed at the far edge of the non-treated cotton area • Dead bee traps (*i.e.*, 5x6 ft piece of fabric) placed on the ground extending out from the hive entrance • A shade structure was erected in each treated plot to provide hives relief from direct sunlight • Trial 3 was irrigated with a center pivot (overhead sprinkler), 0-1.34 inches (total irrigation)			
Lighting:	Natural lighting			
Temperature: Trial 1: May-August 2008 Trial 2: May-August 2009 Trial 3: June-October 2009		Trial 1	Trial 2	Trial 3
	Plot B	--	--	--
	Plot C	--	--	--
	All	63-92°F	65-92°F	58-91°F
Relative Humidity: Trial 1: May-August 2008 Trial 2: May-August 2009 Trial 3: June-October 2009		Trial 1	Trial 2	Trial 3
	Plot B	65-83%	80%	65-86%
	Plot C	65-83%	80%	62-86%
	All	--	--	--
Precipitation: Trial 1: May-August 2008 Trial 2: May-August 2009 Trial 3: June-October 2009		Trial 1	Trial 2	Trial 3
	Plot B	--	--	--
	Plot C	--	--	--

Parameters	Reported Information			
	All	2.03-12.77 in.	2.41-5.99 in.	1.05-8.27 in.
Cloud cover at time of evaluations: Trial 1: May-August 2008 Trial 2: May-August 2009 Trial 3: June-October 2009		Trial 1	Trial 2	Trial 3
	Plot B	40-90%	50-90%	40-80%
	Plot C	40-90%	50-90%	80%
	All	--	--	--

'All' applies to the general testing area, presumably all three plots (low concentration, B; high concentration, C; and, control)
'Plot B' refers to two applications at low concentrations (Trial 1: 7/15/2008 of 40.9 g a.i./A and 7/22/2008 of 40.58 g a.i./A; Trial 2: 7/8/2009 of 40.42 g a.i./A and 7/15/2009 of 40.46 g a.i./A; Trial 3: 8/10/2009 of 41.03 g a.i./A and 8/19/2009 of 40.91 g a.i./A)
'Plot C' refers to two applications at high concentrations (Trial 1: 7/15/2008 of 61.36 g a.i./A and 7/22/2008 of 60.69 g a.i./A; Trial 2: 7/8/2009 of 60.63 g a.i./A and 7/15/2009 of 61.11 g a.i./A; Trial 3: 8/10/2009 of 60.87 g a.i./A and 8/19/2009 of 61.3 g a.i./A)

C. Test Design

<table>
<tr><th>Parameters</th><th>Reported Information</th></tr>
<tr><td>Study site description</td><td>
Location (Trials 1,2): Upper Ty Ty Rd., Tift county, GA, USA 31795
Location (Trial 3): U.S. Hwy 41, Tift county, GA, USA 31795

Planting date: 5/21/2008 (Trial 1); 5/8/2009 (Trial 2); 6/16/2009 (Trial 3)

Planting dimensions: vacuum planter placed 2.5 seeds per foot on a 36-inch row spacing
<table>
<tr><th>Acreage treated</th><th>Trial 1</th><th>Trial 2</th><th>Trial 3</th></tr>
<tr><td>Plot A (control)</td><td>~7</td><td>~4.7</td><td>~8</td></tr>
<tr><td>Plot B</td><td>5.636</td><td>5.58</td><td>5.34</td></tr>
<tr><td>Plot C</td><td>5.580</td><td>5.55</td><td>5.21</td></tr>
<tr><td>Field size</td><td>~24</td><td>~18</td><td>~30</td></tr>
</table>
</td></tr>
<tr><td>Test design</td><td>

3 trials, each with 2 treatments and 1 control field (Plot C)
One treatment received two foliar applications of ~40.5 g a.i./A (Plot B)
Second treatment received two foliar applications of ~60.75 g a.i./A (Plot C)
12 bee colonies randomly assigned to control and treatment groups (4 hives per treatment/control)

</td></tr>
</table>

<table>
<tr><th>Parameters</th><th>Reported Information</th></tr>
<tr><td>Crop description</td><td>• DP 555 / Cotton (Gossypium hirsutum)
• Initial application 2 weeks prior to peak flowering
• Second application 1 week prior to peak flowering
• Addition of hives at peak flowering</td></tr>
<tr><td>Fertilizers/pesticides</td><td>Various applied immediately before and during the test</td></tr>
<tr><td>Plot maintenance</td><td>Based on local practices; Subsoil and lay off rows with uneverferth strip-till</td></tr>
<tr><td>Method of administration:</td><td>Tractor Boom Sprayer (broadcast): Hi Boy open station tractor with a 12-nozzle boom. The target delivery volume was ~7.5 gallons/A</td></tr>
<tr><td>Nominal application rates:</td><td>0 (negative control), 81, and 121.5 g a.i./A [0, 200, and 300 g ai/ha]</td></tr>
<tr><td>Actual application rates:
Rates in g a.i./A. (multiply by 2.471 to get units in g a.i./ha)</td><td>
<table>
<tr><th>Plot</th><th>App.</th><th>Trial 1</th><th>Trial 2</th><th>Trial 3</th></tr>
<tr><td rowspan="2">B</td><td>1st</td><td>40.9</td><td>40.42</td><td>41.03</td></tr>
<tr><td>2nd</td><td>40.58</td><td>40.46</td><td>40.91</td></tr>
<tr><td rowspan="2">C</td><td>1st</td><td>61.36</td><td>60.63</td><td>60.87</td></tr>
<tr><td>2nd</td><td>60.69</td><td>61.11</td><td>61.30</td></tr>
</table>
</td></tr>
<tr><td>Controls:
Negative control and/or diluent/solvent control</td><td>Negative control; no diluent/solvent control</td></tr>
<tr><td>Number of colonies per group:</td><td>4 hives per treatment/control (10 frames per hive)</td></tr>
<tr><td>Solvent:</td><td>N/A</td></tr>
</table>

<table>
<tr><th>Parameters</th><th>Reported Information</th></tr>
<tr><td>Blossom counting:</td><td>None reported.</td></tr>
<tr><td>Climatic conditions:</td><td>Weather was typical for test site and time of year. There was a slow moving two-day (8/22-8/23/2008) tropical storm in Trial 1 for which 7.57 inches of rain was reported; bees are thought to have stayed in the hives during this time.</td></tr>
<tr><td>No. of applications:

Application intervals & dates of application:</td><td>2

7 days
<table>
<tr><th>Plot</th><th>App.</th><th>Trial 1</th><th>Trial 2</th><th>Trial 3</th></tr>
<tr><td rowspan="2">B,C</td><td>1st</td><td>7/15/08</td><td>7/8/09</td><td>8/10/09</td></tr>
<tr><td>2nd</td><td>7/22/08</td><td>7/15/09</td><td>8/19/09</td></tr>
</table></td></tr>
<tr><td>Observations period:</td><td>Multiple times throughout study.</td></tr>
<tr><td>Endpoints assessed:</td><td>Dead bees
Foragers returning to hives
Foraging bees in cotton plots
Mass of hive frames
Number of adults
Number of capped brood</td></tr>
</table>

Evaluations of study endpoints

All hives were exposed to dinotefuran for at least 27 days.

Table 1. Study endpoints

Endpoint	Evaluation
Honey yield (hive health indicator) *Hive vigor*	Mass of each hive frame was estimated at several times after initiation of exposure. Total biomass of hive frames may indicate hive health.
Adult population estimate *Hive vigor*	Mass of adult bees within each hive. Determined by shaking hive frames into a large funnel connected to a bee package container. *This procedure was performed directly after placement on the treatment field, prior to removal from the treatment field, at 42-50 days, and 65-78 days.*
Brood production *Hive vigor*	Counts of sealed broods from digital pictures of one side of each hive frame containing brood. *Pictures were taken directly after placement on the treatment field, prior to removal from the treatment field, at 42-50 days, and 65-78 days.*
Adult bee mortality	Counts of dead worker bees and drones. Determined by examining a 5x6ft piece of fabric placed on the ground at the hive entrance. Measurement is designed to compare relative death rates between treatments. *Counts were made at least eight times during the exposure period; the sheets were observed approximately 2x/week.*
Bee foraging vigor (and relative activity)	Counts of bees entering the hive for 3 min. time intervals per hive. Relative activity of foraging bees per plot (225 ft^2 x 4 reps x 3min) was also determined. *Counts were made at least nine times during the exposure period.*
Spring colony assessment	Overwintering in Georgia. Preparations were made in late fall. Colony health determined with the following parameters (on 2.3.2009, Trial 1; 2.23.2010, Trials 2 and 3): • Presence/absence of a healthy queen • Presence/absence of eggs, larvae • Number of frames per hive containing adult workers • Rank of overall health based on adult numbers (0 = dead, no adults; 1= weak, ≤ 4 frames of adults; and 2 = healthy, >4 frames of adults)

Evaluation of residues

Cotton flower and pollen samples

Flower samples were collected from each trial location at approximately 10-day intervals during the time hives were present in the field; a total of three sample dates were completed (for each trial). Pollen and nectar samples were collected from five randomly selected areas in each plot for a total of 200-250 flowers per plot. Pollen was collected into glass sample jars by shaking flowers into the jar opening. Nectar was collected by returning flower samples to a lab and harvesting nectar with a pipette.

Hive residue samples

Nectar, honey, beeswax, and pollen samples were collected from colonies prior to placement in the trial location, twice during the exposure period (~14 and 28 days after exposure began), and twice during the post exposure period. All samples were kept separate. Within 15 minutes of sampling the sample storage container (plastic bag or bottle) was placed in a container with a coolant followed by storage in a freezer (approx. -20°C) until analysis.

Table 2. Hive residue samples

Sample	Evaluation
Nectar	Collected using a disposable pipette to vacuum nectar from several cells in one frame per hive.
Honey	Collected by using disposable plastic spatulas to scrape honey from capped honey cells in at least two areas of one frame per hive.
Beeswax	Collected by using disposable plastic spatulas to scrape wax from empty cells in at least two areas of one frame per hive.
Pollen	Collected by using disposable plastic spatulas to scoop stored pollen from several cells in one frame per hive.

11. REPORTED RESULTS:

Parameters	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Some supporting field data were not strictly collected according to GLP guidelines (daily weather, historical weather, maintenance chemicals and applications, sample weights, soil data, and irrigation). Records of beekeeping practices were not collected strictly according to GLP guidelines.
Control performance:	Highly variable; refer to Reviewer's Comments for details on specific endpoints.
Raw data included:	Raw data available for honey yield, adult population estimate, and brood production; as well as a qualitative assessment of colonies in the Spring. However, no raw data was provided for adult bee mortality or bee foraging vigor.
Signs of toxicity (if any) were described?	No raw qualitative data included.

Table 3. Adult bee mortality

Number of Observation Dates	Treatment	Cumulative Number of Dead Bees[1] (mean ± SD)	% inhibition
Trial 1			
8	Non-treated	21.3 ±26.2	---
8	40.5 g a.i./A	18.3 ± 6.8	14.1
8	60.75 g a.i./A	14.8 ± 10.1	30.5
Trial 2			
10	Non-treated	53.8 ±44	---
10	40.5 g a.i./A	69 ± 37.7	-28.2
10	60.75 g a.i./A	67.8 ±20.6	-26
Trial 3			
9	Non-treated	39.8 ± 15	---
9	40.5 g a.i./A	17.8 ± 11.7	55.3
9	60.75 g a.i./A	23.5 ± 5.8	41

[1] Sum of all observations per hive for the season.

Note: statistical verification of these results was not possible as the raw data counts were not provided by the study author.

Residue Analysis for dinotefuran only

Trial 1

Flowers

a. Nectar

For the application regime consisting of two applications of Venom® 70SG at a rate of 40.5 g a.i./A, the residue in nectar from flowers was 0.012 mg/kg at 7 days and 0.013 mg/kg at 17 days. For the application regime consisting of two applications of Venom® 70SG at a rate of 60.75 g a.i./A, the residue in nectar from flowers was 0.021 mg/kg at 7 days and 0.019 mg/kg at 17 days. There were indicators of trace residues (<LOQ = 0.006, 0.007 mg/kg for plots B and C, respectively) in flowers collected 27 days post application.

b. Pollen

No residues were observed in pollen ≥LOQ, but values <LOQ (0.008 mg/kg) indicated a possible trace residue in a sample collected from flowers at 17 days post applications (plot B – two applications of Venom® 70 SG at a rate of 60.75 g a.i./A only).

Hives

a. Nectar

Residues from hive-collected nectar samples were <LOQ (0.005-0.009 mg/kg), there were indications of trace residues in nectar collected from hives associated with both treated plots (B and C) at 23 and 34 days post application.

b. Pollen

Values <LOQ (0.004, 0.005 mg/kg) indicated a possible trace residue from hive-collected samples 21 days post application for both treatment groups (B and C).

c. Honey

No residues were observed in honey ≥LOQ, but values <LOQ (0.006, 0.009 mg/kg) at 21 days post application may indicate trace residue for both treatment regimes.

d. Beeswax

Residue values for beeswax were consistently below the LOQ (≤ 0.003 mg/kg).

Trial 2

Flowers

a. Nectar

Measurable residues of 0.047 and 0.115 mg/kg were observed in plots B and C, respectively, at 5 days after the last application. A measurable residue of 0.017 mg/kg was also observed at day 15 for plot C. A trace amount (<LOQ = 0.007 mg/kg) was also observed at day 26 in plot C.

b. Pollen

Trace amounts of dinotefuran residues (<LOQ = 0.0099, 0.009 mg/kg) were observed at day 5 in plots B and C, respectively. For subsequent time steps residue values for pollen were consistently below the LOQ (≤ 0.002 mg/kg).

Hives

a. Nectar

Residue values for nectar in hives were consistently below the LOQ (≤ 0.003 mg/kg).

b. Pollen

Residue values for pollen were consistently below the LOQ (≤ 0.001 mg/kg).

c. Honey

Residue values for honey were consistently below the LOQ (≤ 0.002 mg/kg), except for the untreated plot at day 21 which indicated potential traces of dinotefuran (<LOQ = 0.007 mg/kg).

d. Beeswax

Measurable residue of 0.044 mg/kg was detected in plot C at day 34. The high concentration is attributed by the study authors to contaminated beeswax samples with a material other than wax. Measurable residues of 0.015 and 0.011 mg/kg were observed in the controls at days 21 and 34, respectively. The validity of these controls is unknown. They could arise from cross contamination of samples, mislabeling or result from co-extracted backgrounds. Trace amounts were also detected (<LOQ= 0.008, 0.006, and 0.008 mg/kg) at day 85 in the untreated, B, and C plot samples, respectively.

Trial 3

Flowers

a. Nectar

Measurable residues of 0.065 and 0.101 mg/kg were observed in plots B and C, respectively, at 5 days after the last application. Measurable residues of 0.009 and 0.011 mg/kg for plots B and C at day 15 and a residue of 0.013 mg/kg for plot B at day 26. In addition, the untreated plot sample for day 15 also showed 0.011 mg/kg residue indicating potential cross contamination or sample mislabeling.

b. Pollen

Trace amounts of dinotefuran residues (<LOQ = 0.009, 0.008 mg/kg) were observed at day 5 in plots B and C, respectively. For subsequent time steps residue values for pollen were consistently below the LOQ (≤ 0.001 mg/kg).

Hives

a. Nectar

Residue values for nectar in hives were consistently below the LOQ (≤ 0.002 mg/kg).

b. Pollen
Residue values for pollen were consistently below the LOQ (≤ 0.001 mg/kg).

c. Honey
Residue values for honey were consistently below the LOQ (≤ 0.003 mg/kg).

d. Beeswax
At day 36, residues of 0.013 and 0.028 mg/kg were observed for plots B and C. At day 50, a residue of 0.02 mg/kg was observed for plot C. At day 20, a residue of 0.01 mg/kg was observed in the untreated plot sample.

12. VERIFICATION OF STATISTICAL RESULTS: N/A

13. REVIEWER COMMENTS
The study authors determined that there were no adverse effects detected by any of the measurement techniques employed in this study. However, the EPA reviewer has determined that on account of poor study design and cross-contamination, this study is inadequate to assess toxicity to field pollinators.

14. REFERENCES:

MRID 45639727
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